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SOME STUDIES ON TRYPANOSOMA LEWISI

By LEROY D. SWINGLE

WITH ONE PLATE

During the last four years the literature on trypanosomes has increased marvelously. New species are continually being added to the list, while the morphology and life history of the older forms are being now worked out. Schaudinn's remarkable memoir (1904) on the life cycle of *Trypanomorpha noctuae* and *Trypanosoma ziemanni* has paved the way for the discovery of the life histories of the other forms, and since his work appeared, Prowazek (1905) has discovered that the rat trypanosome passes through stages in the rat louse similar to those found by Schaudinn in the mosquito. It is with the rat trypanosome that the present paper deals.

Woodcock (1906), who has admirably reviewed the present knowledge of haemoflagellates, substitutes the term trophonucleus for nucleus and kinetonucleus for blepharoplast, since the former has to do with nutrition and reproduction, and the latter with locomotion. The fact that the locomotor apparatus develops from the kinetonucleus, as Schaudinn has shown, would seem to justify Woodcock's terminology. For reasons too numerous to give here, he also changes the orientation of the trypanosomes, designating the end formerly considered anterior, as posterior, and *vice versa.* In this discussion I follow Woodcock's terminology, but for reasons which will be given later do not accept his orientation.

The trypanosome of the rat has a geographical distribution coextensive with that of its host. To the recorded places where rats are known to be infected I can also add Seattle, Washington. But, as has already been noted by other authors, the infection in a given city is localized. In Lincoln, Nebr., rats caught at two places a square apart showed infection, while none was found in those from other parts of the city. At one of these centers thirty-six per cent.

of the rats were infected. Adults seemed to be exempt from the disease, but fifty-three per cent. of the rats about half grown and twelve per cent. of those about one-fourth grown were infected. Twenty-three per cent. of all the rats caught were harboring the parasite.

Two field mice and six house mice were examined for trypanosomes, but with negative results. However, the culture method for the detection of the parasite was not used.

A two-thirds grown rat, heavily infected, became sick and died. Several hours afterwards an examination of various parts was made. The parasites were very numerous in the liver, the veins of the fore-limb, and the blood sinuses of the brain. They were less abundant in the spleen, the kidneys, the lungs, and a lymph gland from the axilla. The parasites possessed great activity in spite of the fact that the rat had become very cold and stiff. No hypertrophy of the organs could be detected. The parasites could be found easily in sections of the tissue by staining with carbol thionin. No intracellular stages were discovered.

With regard to technique, iron haematoxylin and eosin give fair results. They bring out the trophonucleus, kinetonucleus, and flagellum plainly. But the trophonucleus takes the stain too readily for the cytoplasm. If the specimen is destained enough to differentiate the chromatin of the trophonucleus, the cytoplasm is not sufficiently stained. Goldhorn's stain was used with mediocre results. But the best differentiation was obtained by the use of Wright's stain. This stain, however, is quite variable, due to the evaporation of the alcohol. For the best results the alcohol must be sufficiently saturated with the stain to give a rich reddish-purple when water is added. In this condition, if the stain be left on the smears from ten to fifteen minutes, the flagellum will take on a deep red color, the cytoplasm a light blue, the trophonucleus a rose-red and the kinetonucleus a darker red. When the parts are thus stained, the connection between the flagellum and kinetonucleus is plainly shown, the flagellum staining right up to the latter.

Authors are very well agreed in regard to the morphology of the adult trypanosome. MacNeal (1904), however, noted that some of the commonly called adults were enlarged and possessed two trophonuclei as late as the one hundred and tenth day of the infection. He explains this as a slow and retarded longitudinal division. Several days after the period of multiplication had passed, I found some parasites with two kinetonuclei and others with two trophonuclei (pl. x, fig. 1). This is not a result of mechanical division of the trophonucleus by smearing, but must be regarded either as a longitudinal division or as an abnormality.

There is considerable disagreement among workers about the mode of multiplication of the trypanosome in the blood of the rat. Rabinowitsch and Kempner (1899) have described three types: longitudinal division, transverse division, and segmentation in which the undulating membrane and flagellum disappear. In their later work (1903) they modified their views, but still held that the formation of rosettes in which the mother cell could not be found does occur. Francis (1903) described and figured the same modes of division as Rabinowitsch and Kempner in their first work. But he says: " These three forms of division are separated only for description. They may all be seen side by side in the same preparation and in some instances the distinction between them is not altogether clear, especially between the longitudinal and transverse modes. Division by segmentation begins with a curving of the parasite which continues until the two ends approach each other and finally meet, thus giving the parasite a globular form. The flagellum becomes lost and the undulating membrane disappears." Laveran and Mesnil (1904) recognized two types of division which they designated as group *a* and group *b*. In the first group the mother trypanosome can be recognized, being larger than the other individuals, while in the second group the individuals are all the same size. Danilewsky distinguished two modes of division, namely, longitudinal division and segmentation. In the latter he says that the flagellum and undulating membrane disappear. MacNeal's observations (1904) agree with those of Wasielewski and Senn (1900), who maintain that unequal longitudinal division is the only method of multiplication.

My observations agree most nearly with those of Laveran and Mesnil. In both living and stained preparations I found each kind of rosette. By an unequal longitudinal division of the mother trypanosome a small cell is formed (pl. x, fig. 3). Several such divisions give rise to a rosette consisting of a number of small, equal cells and the large mother trypanosome (pl. x, fig. 4). Further, each one of the small individuals may divide by equal longitudinal division before separating. In fact small cells divide regularly by this method, and couplets thus produced are found in the blood again and again. And so, when such division proceeds without a complete separation of the individuals, a rosette lacking a large mother trypanosome is formed. A similar rosette would be formed if the mother trypanosome of group *a* should become broken off. Thus, rosettes lacking the mother trypanosome belong to group *b* and are derived from group *a*, as Laveran and Mesnil have already pointed out. A study of rosettes in living preparations gives quite a different idea of their formation from that gained by the study of smears. The individuals do not lie in a single plane, as they are seen in a smear (pl. x, fig. 2), but lie parallel to each other with their flagella all directed the same way (pl. x, fig. 4), unless division has more or less completely taken place. Such rosettes would probably be crushed in spreading and thus give rise to the many fantastic shapes which one always finds in smears. However, if division has proceeded till the individuals are free except at their posterior tips, then in spreading a more or less perfect rosette would be formed (pl. x, fig. 2).

But there is another divisional form which, as far as I am aware, has not been described by any previous author. A one-fourth grown rat was inoculated subcutaneously February 26 with trypanosomes. On March 3 multiplication forms, both large and small, appeared simultaneously. On March 5 the blood showed a heavy bacterial infection which increased till March 7, when the blood presented a very light pink or almost milky color. Not only the tail blood had this appearance, but also the blood taken from the feet. It began to clear up on March 8 and the following day resumed its normal color. The rat died March 11 without showing other symptoms than sleepiness. Examination of the fresh blood revealed peculiar forms, some of which looked as if there was a flagellum at each end, the motion being quite different from the usual character. The true structure of these peculiar forms was not evident, until stained preparations were studied, when it was found that there were two types present. The individuals belonging to the first type are somewhat smaller than an adult trypanosome (pl. x, fig. 5); those belonging to the second type larger than an adult (fig. 6). The forms belonging to these types were quite numerous, not fewer than thirty of each kind having been found in a few smears. They could be picked out very easily, being so similar to each other and so different from the ordinary divisional forms which existed side by side with them (compare pl. x, figs. 6, 7, 8, 10 and 11, also figs. 5 and 9). Although these represent various stages of division, yet it is evident that they all belong respectively to the given types.

The interpretation that should be put on these forms seems doubtful. Evidently the intense bacterial infection was a factor in the cause for their existence, since they did not occur before the appearance of the bacteria. It might be conceived that under this adverse condition to which they were subjected they took to conjugation. But since Prowazek's work on the trypanosome in the rat louse, this interpretation would hardly seem reasonable. The explanation which appears most satisfactory is that they are cases of *equal* longitudinal division, where the flagellum and undulating membrane split their entire length. MacNeal (1904) contends that the flagellum does not divide, but that the new flagellum grows out of the new kinetonucleus in such close proximity to the old one that it appears to split off from it. Laveran and Mesnil (1904) say that the new flagellum is formed by the division of the old one from the kinetonucleus out to the undulating membrane, at which point it breaks out and then lengthens by growth. At any rate, the new flagellum in ordinary cases can be distinguished by the fact that it is shorter and thinner than the old one. But in these cases the flagella are equal in length and thickness, and neither a mother flagellum nor even a mother trypanosome can be distinguished. That the flagellum does actually split is substantiated by many forms (pl. x, figs. 10, 11). In some (fig. 10) the flagellum has split from the kinetonucleus to a point anterior to the trophonucleus, and in others (fig. 11) nearly to the trophonucleus. After the flagellum has split its entire length one can easily see how a bilaterally symmetrical form would result in spreading (figs. 6, 7 and 8). In those cases (fig. 5) where the division of the flagellum seems to have been from the anterior to the posterior, it will be noticed that the kinetonucleus is tardy in division, and we would therefore expect the flagellum to divide in this way, the trophonucleus and anterior cytoplasm having divided before the kinetonucleus. Whatever may be the true explanation of these forms, the fact remains that they are entirely distinct from any form which occurred in the other rats which were inoculated.

Byloff (1904) described and figured a kind of mitosis. Many of my preparations show forms quite similar to his figures, but they occur most abundantly toward the close of the multiplication period and are generally very vacuolate. Moreover, there is not enough definiteness about the arrangement of the chromatin to lead me to regard them as mitotic figures.

In several rats parasites in actual process of division were very scarce in the peripheral circulation, and yet the number of trypanosomes in the blood was increasing rapidly. To be sure, many enlarged forms which were preparing for division and a very few small rosettes were found. Two of the rats were killed and examined. In one rosettes were found to be quite abundant in the spleen; in the other the bone marrow was teeming with them, amply sufficient in number to account for the rapid increase of the parasites in the peripheral blood.

Agglomeration was secured under the following conditions: A one-fourth grown rat was inoculated intraperitoneally with a few drops of blood bearing a rather light infection. Twenty hours later the trypanosomes were found in the blood from the tail and resembled those inoculated. The next day agglomeration began and twenty-four hours later rosettes consisting of as many as sixteen individuals were found. A few days later the parasites had entirely disappeared. No divisional forms were discovered, but, judging from the small number inoculated and the comparatively large number agglomerated, a multiplication must have taken place somewhere in the body. The agglomeration presented some unique forms. Two of the parasites would unite by the posterior and anterior ends, respectively, so that as they moved through the field they gave the appearance of a loop increasing and diminishing with the change in motion of the bodies. Such couples were quite numerous, and were never seen to separate from each other at either end (pl. x, fig. 12).

As MacNeal has observed, the contact of individuals is not merely by their posterior tips, but they overlap. He says that in *Trypanosoma brucei* a perfect fusion takes place, but does not make it clear whether that is the case with *Trypanosoma lewisi.* At any rate, in some cases of the latter a complete fusion takes place and the kinetonuclei come to lie close together or apparently fuse (pl. x, fig. 12). Sometimes, when several are agglomerated, the outlines of the posterior ends are entirely lost and the kinetonuclei all lie close together in a central mass.

As to the mode of transmission, natural infection is doubtless accomplished by the bites of blood-sucking insects, such as lice and fleas. Rabinowitsch and Kempner (1899) succeeded in transmitting the disease by placing on a fresh rat fleas which were taken from an infected one. Similarly, MacNeal (1904) obtained a positive infection by the use of lice. But up to the year 1905 none of the investigators had found other than adult stages in infected lice and fleas. In 1905, however, Prowazek published an account of the life cycle of the parasite observed in the louse. My observations show that it probably passes through a similar cycle in the flea.

Examination of fleas teased in normal salt solution immediately after their removal from an infected rat showed many small oval trypanosomes which had lost their flagella, spindle-shaped forms, and large active ones resembling the adults in the rat. Several with long flagella and with the posterior half of the body bent forward on the anterior part were found. In a flea examined twenty-four hours after it was taken from an infected rat rosettes of four and eight trypanosomes respectively were found. The cells were somewhat thickened and from their motion it was evident that the flagella were centrally located. In another flea two instances were found where agglomeration by the posterior ends had taken place and been followed by a rounding off of the ends, the flagella still persisting. Rosettes of at least seventy-five individuals with their flagella centrally located were found in another flea at the same time.

By sectioning and staining with iron haematoxylin the viscera of the infected fleas, the parasites and their relation to the host could be made out. In the case of a flea which was sectioned forty hours after its removal from the rat very few trypanosomes were found in the stomach, but great masses lined the wall of the rectum. Besides the large masses attached to the wall, here and there small groups were found attached by their flagella (pl. x, fig. 13). Such ones were also found attached to the epithelial cells of the stomach. Rosettes in which the flagella were directed centrally were found free in the rectum (pl. x, fig. 14). Between the epithelial cells of the stomach and in the intestine masses of trypanosomes were found in which the flagella had entirely disappeared and the cells become spherical (pl. x, fig. 15).

In this connection I wish to call attention to two of the reasons why Woodcock regards the non-flagellate end as the anterior. His argument is as follows: When herpetomonadine forms, such as *Trypanomorpha,* enter a resting phase, they attach by the flagellate (anterior) end. Also the heteromastigine form, *Trypanophis,* attaches by the anterior end. But, when *Trypanosoma ziemanni* enters a resting phase, it attaches by the non-flagellate end. Moreover, there are certain other species of trypanosomes which are known to attach to blood corpuscles by the non-flagellate end. Now, since forms in which the orientation is known attach by their anterior ends, he believes that all trypanosomes attach by the anterior end, and since in all cases where the trypanosome is known to attach, it attaches by the non-flagellate end, he would regard this end as the anterior. The second reason is based on the mode of agglomeration. In *Trypanomorpha,* agglomeration takes place by the flagellate (anterior) end. In sharp contrast to this the trypanosomes agglomerate by the non-flagellate end. This end must therefore be the sensitive pole. Since forms in which the orientation is known agglomerate by the anterior end, and since the end by which agglomeration takes place must be considered the sensitive pole, he regards the non-flagellate end of the trypanosome as the anterior.

My observations, however, show that *Trypanosoma lewisi* attaches by the flagellate end (pl. x, fig. 13) and sometimes forms rosettes with the flagella centrally located. Perhaps these rosettes are not agglomerations, but nevertheless the ends directed centrally should be regarded as the sensitive poles as much in this case as in cases of agglomeration. Woodcock gives other reasons which may still justify him in considering the non-flagellate end of trypanosomes as the anterior, but these exceptions would seem to invalidate in a measure his generalizations.

The rosettes found in the flea may be merely cultural forms similar to those found by Novy, MacNeal, and Torry (1906) in the stomachs of mosquitoes fed on infected blood. But in view of Schaudinn's work on the owl trypanosome in the mosquito and Prowazek's work on the life cycle of the rat trypanosome in the louse, it is very probable that other than mere cultural rosettes are to be found in the flea. The discovery of attached forms as already described in this paper seems to confirm this opinion and to demonstrate that the flea also acts as an intermediate host.

A bit of circumstantial evidence to the effect that the flea plays an important rôle in the transmission of the parasite is worthy of mention here. Of the seventeen one-fourth grown rats examined in the autumn and winter not one was infected with either lice, fleas, or trypanosomes, while of the seven caught the following spring at the same place all had fleas and four were harboring the parasite. This was the only place in the city where infection was found to exist, and, moreover, neither lice nor fleas were found on those rats caught in other parts of the city. From this evidence and also from the fact that fleas were found on infected rats much more often than lice, one would conclude that the flea served as the intermediate host more often than the louse. While the life cycle of the parasite in the former is doubtless quite similar to that in the latter, yet an excellent field for further investigation is presented here.

This work was done in the Zoological Department of the University of Nebraska under the direction of Dr. Henry B. Ward, to whom I am indebted for interest and assistance in the investigation.

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[1] Since Laveran and Mesnil (1904) have monographed the trypanosomes, and Woodcock (1906) has given an extended bibliography in connection with his review, there is need to cite only the papers directly bearing on this article.

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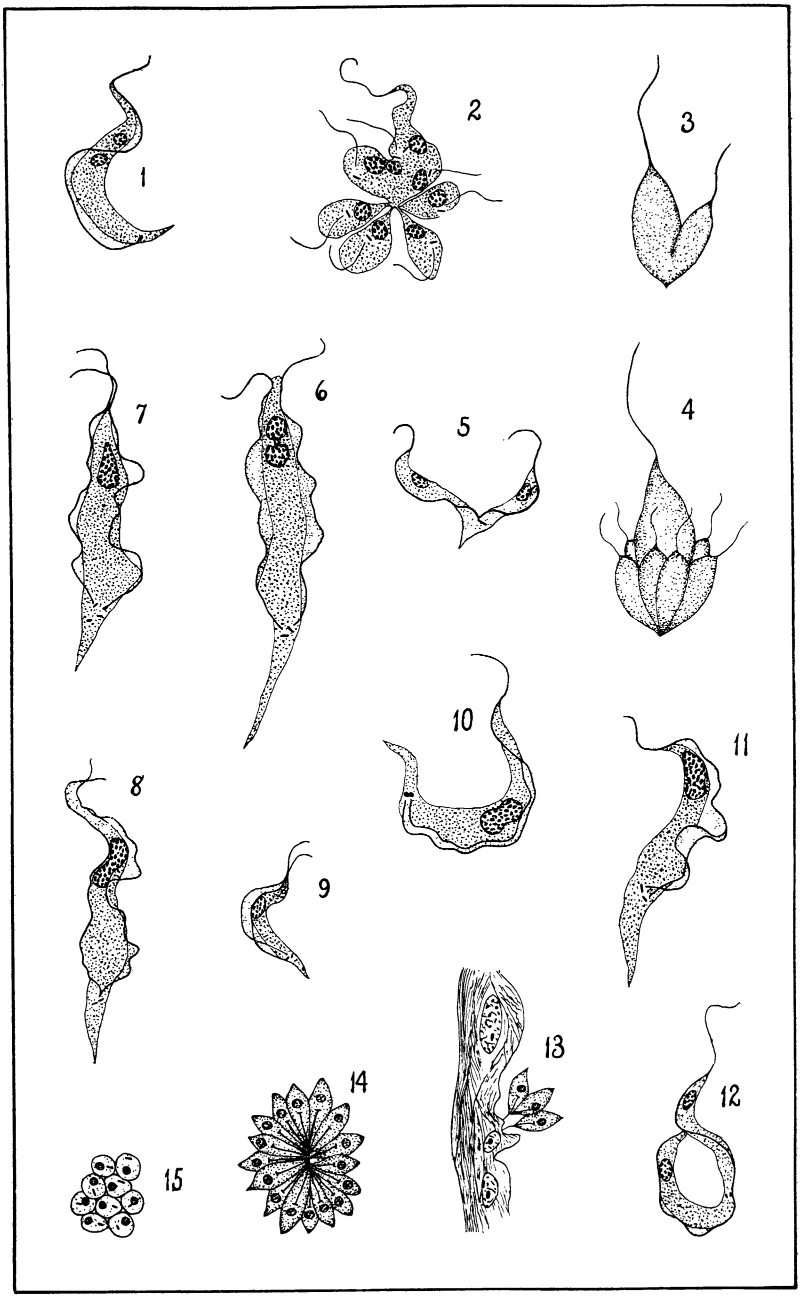
PLATE X



EXPLANATION OF PLATE

Plate X

All figures are magnified approximately 1500 diameters.

FIG. 1. Adult in process of division several days after multiplication period.

FIG. 2. Multiplication rosette showing mother cell and several daughter cells.

FIG. 3. Early stage of unequal longitudinal division as seen in living preparations.

FIG. 4. A later stage than Fig. 3. The cells lie parallel to each other, the anterior ends in the same direction.

FIG. 5. Equal longitudinal division of a form smaller than an adult. The flagellum has split from the anterior to the posterior before the kinetonucleus has divided.

FIG. 6. Equal longitudinal division of a form larger than an adult. It presents bilateral symmetry.

FIGS. 7, 8. Forms corresponding to Fig. 6. In the first, the kinetonucleus has divided twice.

FIG. 9. An earlier stage than Fig. 5. Cytoplasm and trophonucleus not yet divided.

FIGS. 10, 11. Earlier stages than Fig. 6. Flagella in process of division.

FIG. 12. Agglomeration of two individuals by anterior and posterior ends respectively.

FIG. 13. Three trypanosomes attached by their flagella to the wall of the rectum of the flea.

FIG. 14. Rosette free in the rectum. The kinetonuclei lie anterior to the trophonuclei; the flagella are directed centrally.

FIG. 15. Mass of trypanosomes found free in the intestine. The individuals have become spherical and lost their flagella, but retain their kinetonuclei.